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ON THE MODIFICATIONS OF THE METABOLISM PRODUCED BY THE ADMINISTRATION OF DIPHTHERIA TOXINE.

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I. Preliminary.

It is now almost universally admitted that a high temperature is not an essential part of infective febrile processes, but that it, like the other symptoms, is one of the results of the toxic action of the products of micro-organisms, and that it is caused in the first instance by diminished heat elimination. In the later stages of some fevers increased heat production undoubtedly plays a part.

A vast number of observations on the influence of these infective processes on the economy have been recorded, but a study of such a work as von Noorden's Pathologie des Stoffwechsels, shows how much yet remains to be investigated before we can comprehend the modifications in the chemical processes in the body induced by these conditions.

In attempting to gain a knowledge of such changes we have to depend largely on the study of the alterations in the excretions—in the expired air and in the urine.

So far the study of the respired air has yielded more constant and conclusive results than the examination of the urine. For in the case of the latter excretion, when we come to compare the many different observations which have been recorded, the discrepancies which manifest themselves are so evident that it is difficult to draw any satisfactory conclusions.

It appeared to us that a careful and systematic study of the effect

of an infective fever, not merely on one or two, but upon all the important urinary constituents, should assist in the explanation of these discrepancies, and that the comparison of the changes in the several constituents with one another should afford data from which a more satisfactory conception of the modifications in the metabolism might be drawn.

At the same time, it seemed probable that such a systematic study of the influence of a powerful toxic agent upon the metabolism would throw light upon the inter-relations of the various processes by which the urinary constituents are produced.

II. Scope of the enquiry.

With these objects in view we have investigated the influence of fever on the following:—

1. The total excretion of nitrogen.
2. The excretion of nitrogen not precipitated by phospho-tungstic acid. (Urea nitrogen of Bohland.)
3. The excretion of nitrogen as preformed ammonia.
4. The excretion of uric acid nitrogen (in one experiment).
5. The excretion of nitrogen precipitated by phospho-tungstic acid (nitrogen not in urea), determined by the difference between 1 and 2.
6. The excretion of phosphoric acid.
7. The excretion of sulphur, both as sulphates and as neutral sulphur.
8. The excretion of chlorides.
9. The excretion of sodium and potassium.

III. General method of procedure.

Method of producing fever. In the experimental study of the influence of fever Von Noorden and others have used the injection of tuberculin on tubercular subjects. It seemed to us desirable to be able to compare the febrile state with the normal condition, and not with such a pathological state as tuberculosis. For the production of a short sharp attack of an infective fever diphtheria toxine appeared to us eminently suitable, since its action can be so accurately controlled by the use of antitoxine. In using this antitoxine it was of course necessary to ascertain whether it had any influence on the metabolism, and this was done in one of our experiments.

Dogs employed. For such experiments it was impossible to use the human subject. Dogs were consequently employed. These animals have the advantage of resembling the human subject in many points in their metabolism, though—as is well known—they show certain striking differences. They have the advantage of being easily kept upon a fixed diet, of being of suitable size to yield a sufficient quantity of urine for analysis, and of allowing a satisfactory collection of the urine and fæces to be made without any great difficulty. They also react well to the diphtheria toxine.

The plan of the experiments was to put the animal on a fixed diet for several days, until it reached a condition of nitrogenous balance, to then subject it to a one day's fast, and again to put it for three days on the same diet. At the end of this period a dose of diphtheria toxine sufficient to cause a rise of temperature was administered hypodermically, and the animal fasted on the succeeding day. The metabolism in the febrile state could thus be directly compared with the metabolism in simple starvation. The dog was then again put upon the same diet for some days. In the first experiment the febrile day preceded the fasting day. The toxine was kindly supplied to us by Dr Sims Woodhead.

Diet. The animal was fed on porridge made of weighed quantities of Indian meal in the first, second, and third experiments, and of oatmeal in the fourth experiment, with measured quantities of milk. In the second experiment a weighed quantity of liver was added to the food.

Collection of Urine. Female dogs were used, and before the experiment the animal was anæsthetised, the perineum was split, and the mucous membrane stitched to the skin so as to expose the urethral orifice and to allow of easy catheterization. In the first experiment the dog was catheterized at 10 a.m., at 1 p.m., and at 6 p.m. In the second and third experiment it was catheterized at 10 a.m., and at 6 p.m., and in the fourth experiment it was catheterized at 10 a.m. only. The rest of the urine was collected by keeping the dog in a zinc cage with sloping floor terminating in a hole. The dog slept on a smooth board, but passed its urine on the floor of the cage, which was kept scrupulously clean. The urine was collected in a vessel placed below the cage.

Collection of fæces. The fæces in the first three experiments were formed. In the last experiment they were softer. In all the experiments they were removed from the cage as soon after they were

passed as possible, and in no case did they become mixed with urine. They were dried with sulphuric acid in the usual way and preserved for analysis.

IV. Chemical methods.

For the estimation of nitrogen in its various combinations J. C. Dunlop is responsible. For the estimation of chlorine, sulphur, and phosphorus D. Noël Paton is responsible. The determinate of sodium and potassium was carried out by Ivison Macadam.

A. *Urine.* The urine was measured, diluted to a convenient volume and filtered. A sample was preserved in a clean stoppered bottle, so that when necessary any analysis could be repeated. The specific gravity and reaction were noted.

The total nitrogen was estimated by Argutinsky's modification of Kjeldahl's method. Two analyses of each sample were made, and the mean taken.

The nitrogen in urea was estimated by Bohland's method, the nitrogen not as urea being precipitated by phospho-tungstic acid.

The nitrogen not in urea was determined by the difference between the total nitrogen and the urea nitrogen.

The preformed ammonia was estimated by Schlössing's method, 25 c.c. being used.

The uric acid was determined by the method of Hopkins. 100 c.c. were used.

For the chlorides Volhard's method of direct titration with nitrate of silver was used. On several occasions the results were checked by igniting the urine and determining the chlorides in the ash. Concordant results were obtained, as is shown by the following example. 10 c.c. of urine were used.

	Silver solution used in ccm.	
	Before ignition.	After ignition.
Feb. 9.	10·1	10·6
	10·4	
	10·3	
Feb. 10.	1·3	1·7
	1·5	

Phosphoric acid was determined by titrating with uranium nitrate. The method was checked on one or two occasions by the gravimetric method after igniting with caustic soda and nitre. The results were practically the same. 25 c.c. of urine were used.

Sulphuric acid was estimated by heating 50 c.c. of the urine with hydrochloric acid and adding barium chloride. The precipitate was washed and weighed.

The total sulphur was estimated as sulphuric acid by igniting with caustic potash and nitre, dissolving the ash with hydrochloric acid and precipitating with barium chloride.

The sodium and potassium were determined as follows:—

100 c.c. of the urine were acidified with hydrochloric acid and 2 grms. of carbonate of calcium were added. Ammonia was next added and the mixture heated and filtered. Oxalate of ammonia and ammonia were added to the filtrate and it was heated and filtered. The filtrate was evaporated to dryness in a platinum basin and ignited. The ash was dissolved in water and filtered. To the filtrate a solution of strontium chloride in alcohol was added, and it was heated and filtered. The filtrate was then heated to remove the alcohol, and an excess of carbonate of ammonia was added, and it was heated and filtered. The filtrate was dried in a platinum capsule ignited, dissolved in water and the treatment with carbonate of ammonia repeated, and after re-evaporation the residue was dissolved in water, a few drops of hydrochloric acid added, and the fluid was evaporated to dryness in a platinum capsule and weighed. This gives $MgCl_2 + KCl + NaCl$.

The residue was then dissolved in water with a little hydrochloric acid, made up to 100 c.c. and divided into two equal portions *A* and *B*.

In *A* the magnesium was estimated by the phosphate of soda method. From the $Mg_2P_2O_7$ the $MgCl_2$ was calculated and, deducted from the half of the first weighing, gave the $KCl + NaCl$.

To portion *B*, platinic chloride in excess was added—the solution being highly-coloured, by excess of platinic chloride. It was evaporated nearly to dryness in a water-bath and 80 % alcohol was added. After standing for three hours a crystalline precipitate formed. This was washed with a mixture of alcohol and ether. The precipitate was dissolved in hot water, and the process repeated. The precipitate was collected on a dried weighed filter-paper, washed with alcohol and ether, dried and weighed. This gave $2KCl + PtCl_4$. From this KCl was calculated and by subtraction gave the NaCl.

B. *Fæces.* The fæces were weighed, treated with 10 per cent. sulphuric acid and dried.

The nitrogen was determined by Argutinsky's modification of Kjeldahl's method.

Details of experiment.

Two dogs were used, the one for Exps. I. II. and III. the other for Exp. IV. The first dog was a spaniel bitch weighing 9·2 kilos.

Exp. I. Feb. 28th. - The dog was put upon a diet of

Indian meal as porridge	150 grm.
Milk	700 c.c.

On March 4th the diet was increased to

Indian meal	200 grm.
Milk	700 c.c.

On March 9th (sixth day), 12·5 doses of diphtheria toxine (each lethal to 500 grm. guinea-pig) were injected subcutaneously at 3 p.m. On the following day the dog was ill and refused its food. At 4 p.m. it appeared very ill, and unable to rise.

125 immunisation units of antitoxine prepared in the laboratory were injected. At 5·15 the animal seemed better. At 10 p.m. another similar dose of antitoxine was given. Next morning it seemed perfectly well and took food greedily. On March 14th no food was given.

	Temp. in degrees Cent.	
	Morning	Evening
5th day of exp.	38·5	38·6
6th ,,	38·8	38·3
7th ,,	**40·5**	**40·0**
8th ,,	39·5	39·1
9th ,,	39·0	38·4
10th ,,	—	38·5

Weight.

Feb. 28	9·20 kilos.
5th day of exp.	9·77 ,,
7th ,,	8·04 ,,
11th ,,	8·33 ,,
12th ,,	7·68 ,,
26th ,,	9·63 ,,

Food analyses.

Nitrogen.

I. Indian meal (0·5 grm. taken). 40 c.c. $\frac{N}{10}$ acid used, of which 6·2, 6·6, 6·6 and 6·0—average 6·4 c.c.—were neutralised. = 0·089 grm. N. = 1·77 % = **3·55 grm.** in 200 grms.

II. Milk (5 c.c. taken). 40 c.c. $\frac{N}{10}$ acid used, of which 16·6 and 16·9—average 16·75 c.c.—were neutralised. = 0·0234 grm. N. = 0·469 % = **3·28 in** 700 c.c.

Exp. II. On May 1st, the same dog was put on a diet of

Indian meal	100 grm.
Milk	700 c.c.
Minced liver	100 grm.

On May 3rd at 1·30 p.m. 125 immunisation units of antitoxine were injected, and the same dose was repeated at 4 p.m. No symptoms were produced.

	Temperature in deg. C.	
	Morning	Evening
May 3	38·4	38·4
„ 4	37·8	38·4
„ 5	38·4	38·5
„ 6	38·5	38·4

Weight.

May 2.	9·28 kilos.
„ 4.	9·63 „
„ 6.	9·48 „

Exp. III. May 29th. The same dog was put on a diet of

Indian meal	200 grm. (N. 3·56 grm.)
Milk	700 c.c. (N. 3·28 grm.)

On June 3rd (fifth day) the dog[1] got no food.

[1] On May 30th a prolapse of the uterus was noticed and after this date the dog was not catheterized.

On June 6th 18·7 doses of diphtheria toxine (each lethal to 500 grm. guinea-pig) were injected subcutaneously.

On June 7th the dog was ill and took no food.

On June 8th the dog was better, but took only a little food[1], and at 6 p.m. 125 immunisation units of antitoxine were injected.

Temperature.

Between May 30th and June 6th the temperature varied from 38·4 to 38·6° C. On the 7th it rose to 39·5° C. Next day it was 38·9° C. and after it fell to normal.

Exp. IV. Nov. 19, 1898. An old pointer bitch weighing 24 kilos was put on a diet of

Oatmeal	600 grm.
Milk	500 c.c.
Water	500 c.c.

On the 27th (fifth day) no food was given.

On the 30th about 11 doses of diphtheria toxine (each lethal for 500 grm. guinea-pig) were injected at 5·30. On Dec. 1st no food was taken. On Dec. 2 all the food was taken. Next day some of the food was left and the dog seemed ill.

Temperature.

From Nov. 26th to Nov. 30th the temperature varied between 37·8 and 38·4° C. On Dec. 1st it rose to 38·8° C. but in the evening it was only 38·5° C.

Weights.

4th day of exp.	20·9 kilos.
6th ,,	20·7 ,,
8th ,,	20·2 ,,
10th ,,	18·4 ,,
12th ,,	20·2 ,,

[1] Nitrogen of residue = 5·26 grms., so only 1·58 grms. of Nitrogen were taken in food.

General Results.

Exp. I.

Days of exp.		Urine: Quantity	S. G.	Re-action	N Total	N as urea	N not as urea	NH_3	Uric acid	P_2O_5	SO_3	Cl	K	Na	Fæces N	Total N excreted	N of food	N balance
1		1500	1020	alk.	5·505	4·785	·720	·391	·058	·930	·707	11·62	1·662	8·859	·772	6·277	6·83	+ ·56
2	Normal	1960	1015	,,	7·527	6·815	·712	·581	·079	1·248	—	11·48	1·635	6·521	·743	8·270	,,	− 1·44
3		1625	1015	,,	5·956	5·329	·627	·567	·067	1·023	·784	10·11	1·593	5·248	1·140	7·096	,,	− ·26
4		1200	1025	,,	5·880	5·070	·810	·315	·058	1·326	·682	14·20	1·860	8·497	·888	6·768	,,	+ ·07
5		1500	1016	,,	5·835	5·325	·510	·504	·063	·810	·682	10·28	1·037	4·771	1·370	7·105	,,	− ·27
6		1400	1017	,,	5·415	4·757	·658	·510	·058	1·140	·642	9·56	1·419	4·747	·637	6·052	,,	+ ·78
	Average	**1531**			**6·02**	**5·347**	**·673**	**·478**	**·064**	**1·08**	**·70**	**11·21**	**1·534**	**6·607**	**·925**	**6·928**	**6·83**	**− ·1**
7	Injection day	1350	1011	alk.	5·280	4·620	·660	·255	·058	1·20	·688	1·19	1·946	4·768	1·53	6·81	6·83	+ ·02
8	Fever and Fast	140	1027	alk.	2·660	1·995	·665	·085	·034	·42	·151	·28	·42	1·362	·890	3·55		− 3·55
9		1200	1020	acid	8·880	7·675	1·205	·670	·087	1·50	·874	·856	1·104	4·822	·589	9·47	6·83	− 2·64
	Average	**670**			**5·770**	**4·835**	**·935**	**·378**	**·060**	**·96**	**·512**	**·568**	**·762**	**3·092**	**·74**	**6·51**	**3·41**	**− 3·09**
10	Normal	1700	1016	alk.	5·423	4·855	·568	·461	·059	1·131	·734	11·45	1·466	6·093	·218	5·641	6·83	+ 1·19
11		1925	1015	,,	5·660	5·142	·518	·503	·088	1·346	·720	10·05	1·245	5·843	1·300	6·96	,,	− ·13
	Average	**1812**			**5·541**	**4·998**	**·593**	**·482**	**·073**	**1·238**	**·727**	**10·75**	**1·355**	**6·373**	**·759**	**6·3**	**6·83**	**+ ·53**
12	Fast	450	1020	alk.	3·615	3·423	·192	·188	·028	·765	·512	1·46	·631	2·114	·561	4·176		− 4·17
13		1570	1018	,,	5·953	5·314	·639	·327	·061	1·323	·960	11·47	1·212	3·664	·509	6·462	6·83	+ ·37
	Average	**1010**			**4·784**	**4·369**	**·415**	**·252**	**·054**	**1·044**	**·736**	**6·47**	**·921**	**2·889**	**·535**	**5·319**	**3·41**	**− 1·90**

Exp. II.

Days of exp.		URINE: Quantity	Sp. G.	Total N	P_2O_5	SO_3	Cl
1	Normal	2000	1016	6·940	1·60	0·816	6·82
2		1900	1015	6·600	1·52	0·734	4·98
	Average	**1950**		**6·77**	**1·56**	**0·775**	**5·90**
3	Antitoxine	2000	1017	7·280	1·76	0·878	7·16
4		1550	1017	6·664	1·60	0·856	4·67
	Average	**1775**		**6·974**	**1·68**	**0·867**	**5·91**

Exp. III.

Days of exp.		URINE: Quantity	S. G.	N total	N as urea	N not as urea	NH_3	Total S as SO_3	SO_3	P_2O_5	Cl	K	Na	FÆCES N	Total N excreted	N of food	N balance
1	Normal	1750	1015	5·20	4·24	·96	·27		·686	1·00	6·80					6·83	
2		1300	1012	3·86	3·30	·56	·40		·494	·88	4·37					,,	
3		2000	1011	6·50	5·04	1·46	·31		·788	1·16	6·91	2·142	2·308	1·03	7·53	,,	– ·70
4		1650	1012	4·42	3·70	·72	·49	·810	·678	1	—	1·198	4·124	1·03	5·45	,,	+1·38
	Average	**1550**		**5·00**	**4·07**	**·93**	**·37**		**·661**	**1·01**	**6·02**	**1·665**	**3·216**	**1·03**	**6·03**	**6·83**	**+·34**
5	Fast	325	1014	1·71	1·32	·39	·29	·267	·202	·38	·48	·662	·858	·6	2·31		–2·31
6		1300	1014	5·48	4·58	·90	·57	·798	·480	1·16	7·02	1·738	3·670	·92	6·40	6·83	+·43
	Average	**812**		**3·59**	**2·95**	**·64**	**·43**		**·341**	**·77**	**3·75**	**1·200**	**2·264**	**·76**	**4·35**	**3·41**	**–·94**
7	Normal	1250	1012	3·86	3·08	·78	·62	·624	·446	·88	6·91	1·330	3·394	·92	4·78	6·83	+2·05
8		1950	1012	4·88	3·52	1·36	·81	·878	·618	·88	7·28	2·602	4·400	·92	5·80	,,	+1·03
	Average	**1600**		**4·37**	**3·30**	**1·07**	**·72**		**·532**	**·88**	**7·2**	**1·966**	**3·897**	**·92**	**5·29**	**6·83**	**+1·54**
9	Fever	175	1022	1·96	1·46	·50	·38	·281	·113	·52	·46	·658	1·236	·76	2·72		–2·72
10		210	1038	6·02	5·18	·84	·52		·706	·81	·62	1·262	2·264	·76	6·78	1·57	–5·21
	Average	**192**		**3·99**	**3·32**	**·67**	**·45**		**·42**	**·67**	**·54**	**·960**	**1·750**	**·76**	**4·75**		**+3·97**

Exp. IV.

Days of exp.		URINE										
		Quantity	S. G.	N Total	N as urea	N not as urea	NH_3	P_2O_5	Total S as SO_3	Acid SO_3	Neutral SO_3	Cl
1	Normal	2400	1010	10·83	9·15	1·68	1·02	2·76				1·21
2		2990	1009	9·90	9·00	·90	1·32	2·88	2·21	1·31	·90	1·02
3		3055	1010	11·28	8·60	2·68	1·43	3·36	2·52	1·68	·84	·97
4		2595	1009	9·07	7·80	1·27	·87	2·34	2·06	1·23	·83	1·24
	Average	**2760**		**10·27**	**8·64**	**1·63**	**1·13**	**2·83**	**2·26**	**1·40**	**·86**	**1·11**
5	Fast	508	1018	4·65	4·23	·42	·29	·98	1·03	·68	·35	·52
6		2130	1012	11·25	10·41	·84	1·02	3·48	2·22	1·56	·66	·83
	Average	**1319**		**7·95**	**7·32**	**·63**	**·60**	**2·23**	**1·62**	**1·12**	**·50**	**·67**
7	Normal	1950	1010	9·48	7·56	1·92	·98	2·52	1·78	1·29	·49	·40
8		3600	1010	12·20	9·52	2·68	1·76	3·24	2·50	1·56	·94	2·09
	Average	**2775**		**10·84**	**8·54**	**2·30**	**1·37**	**2·88**	**2·14**	**1·47**	**·77**	**1·24**
9	Fever	946	1016	6·10	4·92	1·18	·33	1·34	1·02	·52	·50	·62
10		1235	1020	13·94	11·08	2·86	·97	2·92	2·54	·96	1·58	·50
	Average	**1040**		**10·02**	**8·00**	**2·02**	**·65**	**2·13**	**1·78**	**·74**	**1·04**	**·56**

Consideration of results.

1. *Absorption of Food.*

The experiments recorded by von Noorden (*loc. cit.* p. 208) indicate that in the fever of pneumonia and tuberculosis in the human subject the absorption of proteids and of fats is not interfered with.

In experiments I. and III. the nitrogen of the fæces was determined in the normal period, in the period of fasting (fast and day after), and in the fever period (fast with fever and day after).

Table I. *Nitrogen of Fæces.*

	Normal	Fast	Fever
Exp. I.	0·93	0·53	0·74
Exp. III.	0·97	0·76	0·76
Average	0·95	0·65	0·75

These figures quite clearly show that in the brief fever induced by diphtheria toxine there is no marked interference with the absorption of proteids.

2. *Proteid Metabolism.*

It has already been demonstrated by many observers that one of the chief phenomena of infective fevers is an increase in the proteid metabolism. This as von Noorden points out is not directly connected with the temperature because it manifests itself before the temperature rises, because it may occur when the rise of temperature is prevented by quinine, and because it bears no proportion to the temperature. Like the rise of temperature it is caused by the toxic action of the microbial products which seem, like phosphorus, to cause destructive changes in the protoplasm of the tissues.

So far as we are aware the relative influence of fasting and fever has not been investigated in a case where an accurate nitrogenous balance has been struck.

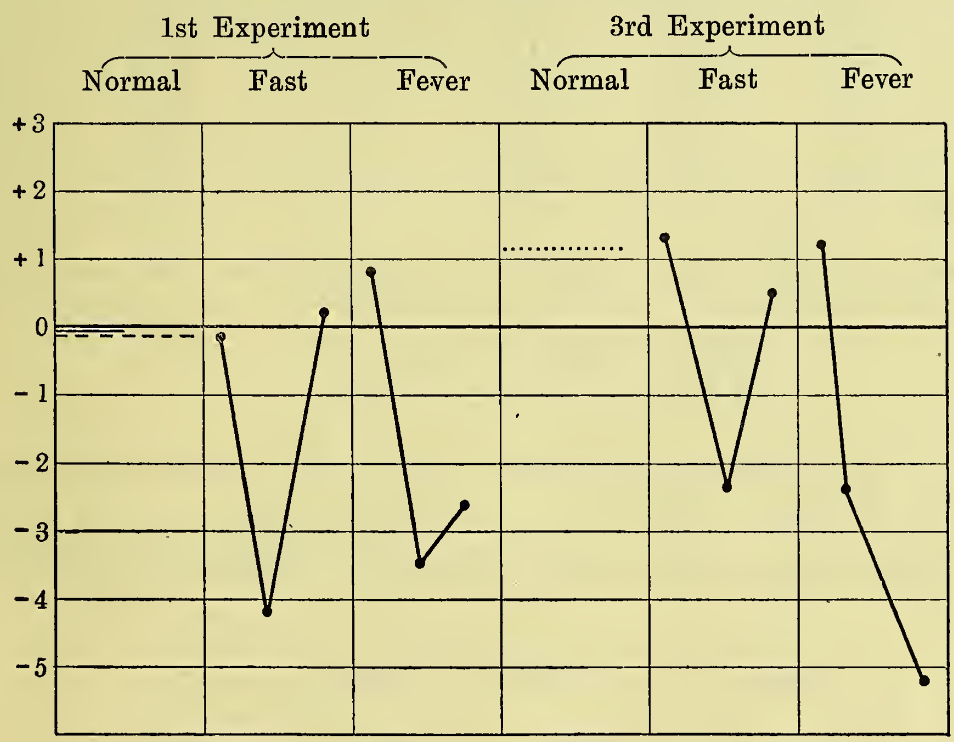
The intake and output of nitrogen in our experiments is shown in the following tables and diagram.

TABLE II. *Intake and output of nitrogen.*

	Exp. I.			Exp. III.			Exp. IV.		
	Food	Urine	Fæces	Food	Urine	Fæces	Food	Urine	Fæces
Normal period	6·83	6·02	0·93	6·83	4·79	·97	—	10·46	—
Fast period	3·41	4·78	0·53	3·41	3·59	0·76	—	7·95	—
Fever period	3·41	5·77	0·74	0·78	3·99	0·76	—	10·02	—

In the diagram the condition of nitrogenous equilibrium is represented by the heavy line. All above this indicates the retention of nitrogen, all below a loss of nitrogen from the tissues in excess of the nitrogen taken in. The normal period is represented by an interrupted line. The fast, and fast with fever, periods are represented by three dots, the first being the day previous to the fast or fast and fever, the second the day of the fast or fast and fever, and the third the day after each of these.

Scheme showing nitrogen balance.



There was thus a marked difference between the effect of fasting, and of fasting combined with fever. The effect of one day's fast was to cause a decided fall of the nitrogen balance. In the first experiment the balance was reduced by 4 grammes, in the third by 3 grammes, but in

both cases this large negative balance was immediately recovered from and followed by a positive balance. When fever was combined with fasting there was a similar drop on the fasting day, but on the following day, instead of recovery with a positive balance, there was a continued negative balance. It is this second day of negative balance which marks the difference between fasting and fasting combined with fever.

Table II. shows that the increased excretion of nitrogen in fever is due to the increase in the nitrogen of the urine. The absolute increase in each case was 0·99 grm., 0·4 grm., and 2·07 grm. These amounts indicate an increase in fever of 21 %, 11 % and 26 %—average 21 %—over the excretion during simple fasting.

3. *Distribution of Nitrogen in the Urine.*

A. Urea.

TABLE III. *Nitrogen excreted as urea and Nitrogen not as urea.*

	Normal		Fast		Fever	
Exp.	as urea	not as urea	as urea	not as urea	as urea	not as urea
1	5·35	0·67	4·37	0·42	4·83	0·94
3	3·83	0·97	2·95	0·64	3·32	0·67
4	8·60	1·85	7·32	0·63	8·00	2·02
Average	5·93	1·16	4·88	0·56	5·38	1·21

Of the excess of 1·15 grms. of N excreted in fever as compared with fasting

0·50 grm. were in urea
0·65 grm. were not in urea.

Thus, of the increased excretion of nitrogen in fever rather less than half is urea nitrogen, rather more than half is nitrogen not in urea.

The percentage of the total nitrogen in urea is given in the next table.

TABLE IV. *Percentage of nitrogen in urea.*

Exp.	Normal	Fast	Fever
1	89	91	84
2	80	82	83
3	82	92	80
Average	84	88	82

A comparison of this with the results obtained by Gumlich and Bohland in their observations on the human subject in fever are of interest.

TABLE V.

	Normal		Fever	
	urea N	not urea N	urea N	not urea N
Gumlich	86·4	13·6	84·5	15·5
Bohland	85·0	15·0	82·0	18·0
Present exp.	84·0	16·0	82·0	18·0

Our experiments further show that in fasting as compared with a diet of meal and milk, the proportion of nitrogen in urea is increased.

B. Ammonia.

In fever in man all observers record an increase in the excretion, both absolute and relative, of nitrogen in ammonia. This von Noorden (*loc. cit.* p. 210) connects with the increased formation of acid by which the alkalinity of the blood is markedly lowered. The amount of preformed ammonia in our experiments is given in the following table.

TABLE VI. *Average daily amount of preformed ammonia.*

Exp.	Normal	Fast	Fever
1	0·48	0·25	0·38
3	0·65	0·43	0·45
4	1·23	0·60	0·65
Average	0·75	0·43	0·49

Expressed as nitrogen this gives

TABLE VII.

Exp.	Normal	Fast	Fever
1	0·40	0·21	0·31
3	0·53	0·35	0·37
4	1·01	0·48	0·54
Average	0·65	0·35	0·41

The percentage of ammonia nitrogen to the total nitrogen was

TABLE VIII.

Exp.	Normal	Fast	Fever
1	6·6	4·4	5·4
2	11·0	9·7	9·3
3	9·6	6·0	5·3
Average	9·1	6·7	6·6

The absence of an increase in the ammonia nitrogen in these experiments is of great interest, but the probable explanation will be dealt with when the excretion of sulphur is considered.

C. Uric Acid.

In the dog the amount of uric acid in the urine is very small, and the influence of fever upon its production cannot be satisfactorily studied. This is to be regretted because most of the observations on man have been made with unsatisfactory methods, and the increase in the excretion of uric acid commonly described cannot be considered as definitely demonstrated.

In our series of experiments the uric acid was determined only in the first with the following results:—

TABLE IX. *Excretion of uric acid.*

Normal	Fast	Fever
·06	·054	·06

Not merely the variations in the uric acid but the total amount determined in 100 c.c. of the urine, the quantity used for analysis, fell within the limits of experimental error and the investigation was therefore not continued.

D. Nitrogen in other combinations.

This includes the nitrogen in such compounds as creatinin, purin bases, cynurenic acid, hippuric acid, etc. Weil and Anrep have shown that in septic fever in rabbits the formation of hippuric acid is interfered with, the benzoic acid of the food being in great measure excreted as such.

The excretion of creatinin has not been fully investigated[1]; but the

[1] See von Noorden, *loc. cit.* p. 213.

evidence is in favour of the occurrence of an increased excretion of this substance in connection with the increased katabolism of the muscle protoplasm.

We are not aware of any investigations on the influence of fever on the excretion of cynurenic acid or upon the production of the purin bases.

In our observations it was impossible to deal in detail with all these various bodies. Their collective quantity however is indicated by the amount of nitrogen excreted in the urine it forms other than urea and ammonia, and this is shown in the following table.

TABLE X. *Amount of nitrogen not in urea.*

Exp.	Normal	Fast	Fever
1	0·67	0·42	0·94
3	0·97	0·67	0·67
4	1·85	0·63	2·02
Average	1·16	0·56	1·21

The table shows a marked increase in the excretion of nitrogen *not* in urea, and as the ammonia excretion is not increased, this must be due to an increase in the excretion of some or all of these combinations.

4. *Excretion of sulphur.*

In previous experiments a general relationship between the excretion of sulphur in the form of sulphuric acid and the excretion of nitrogen has been observed. But this relationship is far from being a fixed one. According to von Noorden the divergencies recorded may in part be due to the excretion of nitrogen and sulphur not necessarily occurring at the same time, and in part to some alteration in the proportion of sulphur excreted as sulphuric acid, and that excreted in such neutral compounds as cystin, etc. The only investigation in any way bearing upon this subject with which we are acquainted is by Schmidt[1]. He shows that in actinomycosis there is an increase in the proportion of sulphur in neutral compounds. He had previously discovered the same condition in pernicious anæmia.

The question is one of very considerable interest, since such a change must indicate a profound disturbance in the metabolic processes.

[1] *Centrbl. f. innere Med.* XIX. 8. 1898.

For its investigation the dog is eminently suitable since a large proportion of the sulphur of the urine is in such neutral combinations.

TABLE XI. *Excretion of* S *in sulphates expressed as* SO_3.

Exp.	Normal	Fast	Fever
1	0·70	0·74	0·51
3	0·62	0·34	0·42
4	1·43	1·12	0·74
Average	0·91	0·73	0·56

The excretion of the total sulphur throughout an entire experiment was studied in Exp. IV. The following results were obtained.

Exp.	Normal	Fast	Fever
4	2·21	1·62	1·78

In Exp. III it was investigated only on the day before the fast, on the fasting day, during the mid normal period, and on the day of fever. Taking the fasting day alone and the fever day alone in these experiments it is found that percentage of sulphur in acid compounds is as follows—

TABLE XII. *Per cent. of* S *in acid compounds expressed as* SO_3.

Exp.	Fast	Fever
3	75	40
4	45	24

These results indicate a marked influence of the febrile process upon the distribution of sulphur in the urine, and this is further elucidated by a study of the relationship of the excretion of sulphur to the excretion of nitrogen.

TABLE XIII. SO_3 *per cent. of total* N.

Exp.	Normal	Fast	Fever
1	12·3	15·4	8·7
3	12·7	9·2	10·5
4	20·8	20·3	17·8
Average	15·2	14·9	12·4

TABLE XIV.

S *in neutral combinations expressed as* SO_3 *per cent. of total* N.

Exp.	Fast	Fever
3	3·9	8·5
4	1·1	1·7

During the febrile process there appears to be a less complete oxidation of sulphur than in the normal or in the fasting state.

Taken along with the diminished elaboration of nitrogen into urea this seems to indicate a profound alteration in the metabolic processes. The part played by the liver in the formation of urea suggests that it is the chemical changes in this organ which are at fault. And this idea appears to be supported by the facts that in fever the true bile constituents are diminished[1] and that the accumulation of glycogen in that organ is also interfered with[2].

This diminution in the formation of sulphuric acid probably explains the absence of an increase in the ammonia of the urine in these febrile attacks. The object of the formation of ammonia appears to be to neutralise the acids and chiefly the sulphuric acid formed in the metabolism of proteids. As before stated this increase is very marked in the human subject in febrile condition, but in the dog under the influence of diphtheria antitoxine it is absent.

5. *Excretion of phosphorus.*

In the various investigations on the influence of fever on the excretion of phosphoric acid very contradictory results have been obtained[3] and the subject requires re-investigation.

In our observations the excretion of P_2O_5 was as follows:

TABLE XV. *Excretion of* P_2O_5.

Exp.	Normal	Fast	Fever
1	1·08	1·04	0·96
3	0·97	0·77	0·63
4	2·85	2·23	2·13
Average	1·63	1·35	1·24

The chief point of interest in the excretion of P_2O_5 is its relationship to the excretion of N., since it has recently been maintained that an increased proportionate excretion of P_2O_5 indicates an increased metabolism of tissues rich in nuclein compounds. The old researches of Zuelzer[4] had shown that in feeding with kidney and liver the

[1] *Reports from the Laboratory of the Royal College of Physicians, Edinburgh*, Vol. III. p. 209. 1891.

[2] *ibid.* Vol. v. p. 75. 1895.

[3] See von Noorden, *loc. cit.* p. 213.

[4] *Semiologie des Harns*, 1884.

proportion of P_2O_5 to N. in the urine rises as compared with the excretion on a diet of flesh. In the former the amount of nuclein is small while it is much larger in the latter.

TABLE XVI. P_2O_5 *per cent. of total* N.

Exp.	Normal	Fast	Fever
1	17·9	21·9	16·8
3	20·5	21·6	15·8
4	27·2	28·0	21·3
Average	21·9	25·7	17·9

The febrile process in all these experiments caused a fall in the proportion of P_2O_5 to N. This appears to indicate either that the tissues specially rich in nucleins are less rapidly decomposed than those poor in these constituents, or that there is some arrangement by which the phosphorus is retained in the body.

This decrease in the proportion of P_2O_5 along with the increase in the nitrogen not as urea—which of course includes the purin bases—would seem to afford further evidence that these are not entirely derived from the nucleins but, as is indicated by the researches of Hopkins, that they may have other sources in the animal body.

6. *Excretion of sodium and potassium.*

The only study of the influence of fever on the excretion of sodium and potassium was made by Salkowski in 1871[1].

By observations on himself and upon others on ordinary diets he showed that the potassium usually forms about 20 or 30 % of the combined sodium and potassium. This proportion he found is increased both by fasting and by fever. For in a case of partial inanition it rose to 40·8 % and in feverish conditions, such as pneumonia and typhoid fever, potassium in some cases constituted as much as 90 % of the combined alkalis.

The results of our observations are given in the following table:—

TABLE XVII. *Excretion of sodium and potassium.*

Exp. I.

	K	Na	K+Na	K per cent. of K+Na
Normal	1·53	6·61	8·14	18·8
Fast	0·92	2·89	3·81	24·2
Feve	0·76	3·09	3·85	20·0

[1] *Virchow's Arch.* LIII. p. 209. 1871; and LVIII. p. 391. 1882.

Exp. III.

Normal	1·80	3·55	5·35	33·6
Fast	1·20	2·26	3·46	34·7
Fever	0·96	1·75	2·71	38·1

A study of these figures shows that, as might be expected, the amount of sodium varies with the amount of salt in the food. In the first experiment the amount excreted per diem was 6·61, while in the third experiment it was only 2·9 gr. On the other hand the amount of potassium was more constant—1·53 in the first and 1·80 in the third experiment.

The figures also clearly show that the effect of fasting and of fasting with fever is to reduce the amount both of sodium and potassium excreted, but that neither in fasting or in fasting with fever is there any marked change in the relationship of the one base to the other.

Various explanations of the discrepancies between our results and those of Salkowski present themselves for consideration.

1. It might be urged that in the transitory attacks of fever induced by our method, the full effect of the toxine had not manifested itself. In Salkowski's cases the fever had been in progress for some time. But the distinct effect produced upon the excretion of nitrogen, upon the distribution of sulphur, and on the amount of chlorine excreted, appear to us to show that the toxine had in our experiments fully exercised its influence on the metabolism.

2. Salkowski's results may have been due to continued starvation rather than to the action of the toxine. But the observation of Munk upon Cetti which show that even upon the tenth day of complete inanition the potassium constitutes only 70 % of the total alkalis, while in Salkowski's cases it sometimes reached over 90 %, appears to militate against this explanation. In muscle alone of the tissues does the percentage of potassium amount to 90 % of the total alkalis. The fact that the increase in the proportion of potassium is largely due to the diminution in the excretion of sodium appears rather to suggest that Salkowski's results may have been due to a retention of the latter base, possibly connected with the fall in the excretion of chlorine presently to be considered.

3. It may be that the action of diphtheria toxine upon the metabolism is different from the action of the toxines of pneumonia or of typhoid.

4. It is possible that the different distribution of the two alkalis in

man and in the dog may in part at least explain these discrepancies. In man the amount of potassium is large in the red blood corpuscles relatively to the sodium, while in the dog this is not the case.

Per cent. of alkalis in ash of red blood corpuscles.

	K	Na
Man	40·89	9·71
Dog	6·07	36·17

Since in starvation the red corpuscles are constantly being broken down, it is possible that the absence of any increased proportion of potassium in the urine may be explained in this way.

7. *Excretion of chlorine.*

Since Redtenbacher[1] observed that, in the course of pneumonia, the excretion of chlorides in the urine sinks, a very large number of investigations on the influence of febrile processes on the excretion of these substances have been recorded. The most recent of these is by Hutchinson[2].

He confirms the results of previous investigators not only as to the influence of pneumonia and many acute fevers on the excretion of chlorine but also as to its cause. He shows that it is not due to a diminished intake or absorption, nor to a failure in the action of the kidneys, but rather to an accumulation of chlorine in the solid tissues. It has been suggested that for the increased metabolism of fever a larger amount of chlorine is required in the tissues, but this is no more than a theory.

In our experiments the amount of NaCl in the food was different in each. In the first 18 grm. per diem was given; in the third 10 grm.; and in the fourth none. These are the weights of ordinary commercial salt added to the food. Our results are given in the following table:—

TABLE XVIII. *Excretion of chlorine per diem.*

Exp.	Normal	Fast	Fever
1	11·21	6·47	0·56
3	6·50	3·75	0·54
4	1·15	0·67	0·56
Average	6·29	3·63	·52

[1] *Zeitsch. d. k. k. Ges. d. Aerzte zur Wien*, VI. p. 373. 1850.
[2] *Journal of Pathol. and Bacter.* p. 406. 1898.

In the first experiment a marked fall in the excretion of chlorine took place even on the day of the injection.

These three experiments show that the influence of diphtheria toxine is to diminish the excretion of chlorine in the urine, the effect being most marked in the first and third experiments when salt was given with the food.

An enormous diminution in the excretion of chlorine as compared with the proteid waste is also manifest.

TABLE XIX. Cl *per cent. of total* N.

Exp.	Normal	Fast	Fever
1	186	135	9·7
3	135	104	14
4	11	9	5
Average	106	81	32

Of much greater interest is the relationship of the excretion of chlorine to the excretion of the alkalis. It is usually supposed that the greater part of the sodium of the urine is linked with chlorine as chloride of sodium. But while this may be so in normal conditions it is not so during fasting and still less during fasting with fever.

The following table shows the amounts of sodium and potassium uncombined with chlorine. In making these calculations it is supposed that the sodium is first all combined.

TABLE XX. *Percentage of* Na *and* K *combined with* Cl.

	Na			K		
	Normal	Fast	Fever	Normal	Fast	Fever
1	100	100	8·5	28	100*	0
3	100	100	19	37	24	0

* Excess of chlorine 0·89 grm.

From this table it will be seen that in the normal and fasting periods, there is sufficient chlorine in the urine to combine with all the sodium and a considerable proportion of the potassium; but that during fever the chlorine is sufficient to combine with only a small part of the sodium and with none of the potassium.

The fever induced by diphtheria toxine thus seems to cause a very marked and special disturbance in the relationship of the chlorine and the alkalis of the urine.

Very similar results were obtained by Salkowski, although in his paper[1] he does not consider the excretion of chlorine in relationship to the excretion of the alkalis. But we find two cases in which the excretion of chlorides was studied and we here reproduce his figures, converting the NaCl into Cl.

From pp. 218 and 225.

	K	Na	K+Na	Cl	Temperature
24	3·044	0·354	3·398	1·085	36° to 40° C.
25	2·301	0·311	2·612	1·221	
26	1·701	0·226	1·927	1·241	
27	2·629	0·521	3·150	1·524	
28	2·438	0·518	2·956	1·599	
29	1·906	0·360	2·266	1·566	
30	0·862	0·625	1·487	1·417	36° C.
31	1·032	3·984	5·016	7·87	below 36° C.
1	2·905	7·540	10·645	16·08	

From pp. 224 and 225.

	K	Na	K+Na	Cl	Temperature
21	3·44	0·77	4·21	1·45	above 40° C.
24	0·520	2·934	3·454	5·106	between 36 & 37° C.
25	0·728	4·334	5·062	8·74	
26	2·260	6·431	8·691	15·58	below 36° C.
27	1·528	3·206	4·734	9·24	

The meaning of this condition is not easy of explanation. It may indicate a retention of chlorine as some halogen compound of the proteids, but our investigations throw no light upon this question. Nor do they elucidate the problem of what acids take the place of hydrochloric acid in combining with the alkalis in the urine. It is known that in fever lactic acid and other organic acids appear in the blood and it is possible that these may replace the chlorine.

Conclusions.

Under the influence of diphtheria toxine in the dog—

There is no interference with the digestion and absorption of proteids.

There is a marked increase in the proteid katabolism, probably as a result of a direct toxic action on the protoplasm.

There is an interference with the elaboration of waste nitrogen into urea which leads to an increase in the proportion of nitrogen not as urea in the urine.

No marked increase in the proportion of nitrogen in ammonia occurs.

[1] *Virchow's Arch.* LIII. 1871.

The excretion of uric acid is not manifestly modified.

The excretion of sulphuric acid is not increased proportionately to the increased excretion of nitrogen.

The total excretion of sulphur bears a fairly direct proportion to the excretion of nitrogen.

The neutral sulphur—sulphur not as sulphates—is increased, probably by an interference with its oxidation to sulphuric acid.

The absence of an increase in the formation of sulphuric acid probably explains the absence of an increased formation of ammonia.

The diminished elaboration of sulphur into sulphuric acid appears to be connected with the diminished elaboration of nitrogen into urea. Both are probably due to an interference with the hepatic function similar to that already demonstrated to occur in febrile conditions in the manufacture of the bile constituents.

The excretion of phosphorus is not increased but is lower in fasting with fever than in fasting alone.

The proportion of phosphoric acid to nitrogen is markedly diminished. This would seem to indicate the absence of any increase in the katabolism of nucleins as compared with other proteids.

The absence of any relationship between the phosphoric acid and the nitrogen not as urea seems to militate against the view that the purin bases which contain part of that nitrogen are necessarily derived from nucleins.

There is no marked alteration in the proportion of potassium and sodium such as was observed by Salkowski in febrile conditions in man. This may possibly be explained by the different distribution of the two bases in the red blood corpuscles of man and of the dog.

The excretion of chlorine is markedly diminished actually, in proportion to the excretion of nitrogen, and most markedly in proportion to the excretion of sodium and potassium.

This alteration in the proportionate excretion of chlorine and of these bases raises the question of what is the nature of the chlorine compounds of the tissues and suggests that in the body chloride of sodium may be split up elsewhere than in the gastric mucosa.

The question of what acid takes the place of hydrochloric acid in combining with the bases in the urine is not elucidated.

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